

## STARCH HYDROLYSIS

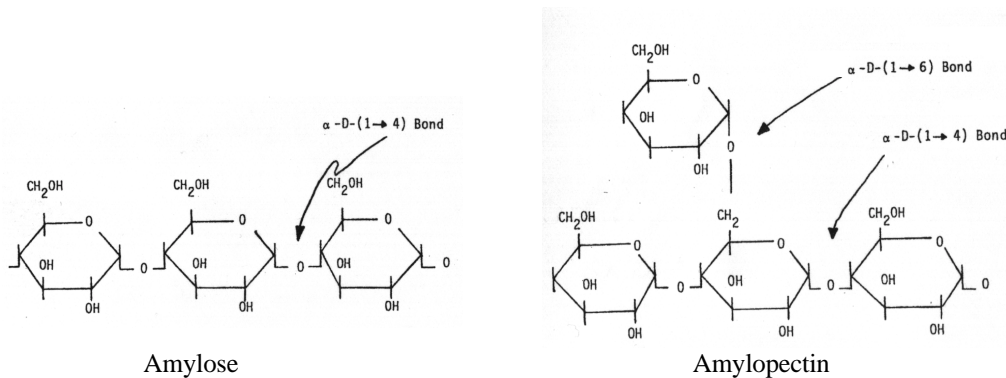
The cornstarch separated from the kernel by the wet milling process is generally 99% pure and contains 0.25-0.35% protein, 0.5-0.6% lipid and less than 0.1% minerals. 35% of the industrially prepared cornstarch is utilized by the food industry; the remainder of the starch is further refined or modified for use in the paper and construction industries. A significant proportion of the cornstarch derived from the wet-milled process used for food goes into the fermentation of beer. To be useful to the breweries, it first has to be converted into dextrose or d-glucose. This is an example of down-stream processing; a raw material is isolated and used as a starting material in another process.

## STARCH FOUND IN CORN

Cornstarch is found in granules within the kernel as a long polymer composed of two structural classes: amylose and amylopectin. The basic repeating unit for both types of starch is d-glucose molecules, connected by glycosidic bonds. The polymer chains and the formation of the intermolecular network traps water and results in gel formation and solution thickening. After the starch is completely hydrolyzed or broken down its basic component is d-glucose also called dextrose or corn sugar.

## MOLECULAR STRUCTURE OF STARCH

Amylose is a linear polymer of short 1,4 linked glucose chains. Typically the amylose fraction is about 25-30% of the starch molecules found in corn and has a molecular weight of about 250,000. The percentage of amylose in the starch is genetically determined. Genetic modifications producing high-amylose (50-70%) cornstarch are also found. Amylopectin comprises about 70-75% of the starch found in the corn kernel and has a molecular weight of about 50-500 million. Amylopectin is a branched polymer of the basic repeating units of 1,4 linked glucose with branches of 1,6 linked glucose. The branching occurs irregularly in the starch, approximately one per twenty-five glucose units.



## AMYLASES

Amylases are a class of enzymes that are capable of digesting these glycosidic linkages found in starches. Amylases can be derived from a variety of sources. Amylases are present in all living organisms, but the enzymes vary in activity, specificity and requirements from species to species and even from tissue to tissue in the same organism. Alpha-amylase (1,4  $\alpha$ -D-Glucan-glucanohydrolase) acts upon large polymers of starch at internal bonds and cleaves them to short glucose polymers.  $\alpha$ -amylase catalyzes the hydrolysis of internal  $\alpha$ 1-4 glucan bonds in polysaccharides containing 3 or more  $\alpha$ 1-4 linkages; it results in a mixture of maltose and glucose. Amyloglucosidase works on the shorter polymers and splits off single glucose sugars. Bacterial  $\alpha$ -amylase is particularly suited for industrial usage since it is inexpensive and is thermally stable.

## ASSAY OF THE AMOUNT OF STARCH IN CORN FRACTIONS

It may be desirable to determine the amount of starch in each fraction of the corn kernel to determine the efficiency of the fractionation process and to determine the suitability of the particular batch of corn or the genetic makeup of the particular corn strain. Therefore a simple assay for the presence and amount of starch is possible. The assay that is used is based on the hydrolysis of the starch by the above enzymes. The final product of the hydrolysis, glucose, is then determined by the glucose oxidase assay.

## DETERMINATION OF THE AMOUNT OF GLUCOSE PRESENT

Glucose is determined by an enzymatic reaction. The enzyme glucose oxidase converts glucose to gluconic acid and hydrogen peroxide in equal molar amounts. The amount of glucose is then determined by the reaction of the peroxide with the chromophore, aminoantipyrine, to produce a color change that is detected at 505nm.

## SYNOPSIS

Sample is prepared and primary digestion with  $\alpha$ -amylase  
Secondary digestion with amyloglucosidase results primarily in glucose  
Glucose is digested with Glucose oxidase to gluconic acid and hydrogen peroxide  
Hydrogen peroxide is changed to an active form by hydrogen peroxidase  
The product of the peroxidase reaction complexes with the chromophore, aminoantipyrine  
Color change is followed by change in absorbance at 505nm

## SAFETY

Read the MSDS information for all chemicals used in this exercise. Phenol and aminoantipyrine are considered harmful or hazardous materials, use gloves when using these compounds. Wear lab coat and proper eyewear.

## MATERIALS

cornstarch (from commercial source or use the cornstarch isolated from Corn Wet Milling Lab)  
bacterial  $\alpha$ -amylase (Sigma A-3403)  
amyloglucosidase (Boehringer Mann 846)  
glucose oxidase (Sigma G-6500)  
anhydrous glucose (Sigma G-5000)

### Glucose oxidase-Peroxidase reagent (GOP)

$\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$  11.5g  
 $\text{K}_2\text{HPO}_4$  2.5g  
Phenol 500mg  
4-aminoantipyrine 75mg (Sigma A4382)  
Peroxidase 3500U  
Glucose oxidase 3500U

Dissolve in water and make up to 500mL. pH the solution to 7.0 and filter through a glass microfiber filter (Whatman GF/A)  
Store at 4 ° protected from light and use within one month

### Amyloglucosidase (AMG) 2mg/mL

Amyloglucosidase 30mg  
dissolve in 15mL of AMG buffer

### AMG Buffer(pH 4.6)

Citric Acid monohydrate 460mg  
Sodium citrate dihydrate 840mg

Dissolve in 100mL water check pH to 4.6

## PREPARATION OF SAMPLE

Weigh samples of 50-100 mg starch and place in 10mL tubes.

Add 2mL of water and 0.2mL of  $\alpha$ -amylase to each tube

Mix immediately on vortex mixer

Add 6 mL of water and mix by repeated inversions

Immerse the tubes in a water bath 85° with shaking for 3 minutes make sure that there is a rapid gelatinisation

Keep at 85° with occasional shaking for 30 minutes

Cool to 20°

Dilute to a total volume of 100 mL

## DIGESTION WITH AMG

Place the following in screw capped tubes that have a tight fitting seal

1) Prepare blank

Add 0.2 mL of  $\alpha$ -amylase to 100 mL of purified water

2) Prepare Glucose standard

make up fresh 1mg/mL glucose standard from anhydrous glucose

3) Prepare samples

Take 1mL of the above prepared sample or dilution of above in purified water

	adjusted volume	AMG	AMG Buffer
Blank $\alpha$ -amylase	1 mL.	1 mL.	---
Glucose @ 1mg/mL	1 mL.	---	1 mL.
Samples	1 mL	1 mL	---

Mix gently

Incubate in a 60° water bath for 30 minutes

Cool to 20°

Add exactly 8 mL of water to each of the tubes

Filter the samples through Whatman glass fiber filter (GF/A)

Take a total of 1mL from the AMG digestion for analysis of total glucose

## ANALYSIS OF TOTAL GLUCOSE

1) Prepare blank

Take 1 mL of the digested blank ( $\alpha$ -amylase)

2) Prepare glucose standards

From the digested 1mg/mL make dilution to cover the range 50-100ug

3) Prepare the samples

Take samples from the above AMG digestion make dilutions where appropriate

Analyte	volume	water	GOP
Blank water	---	1 mL.	5 mL.
Blank $\alpha$ -amylase	1 mL.	---	5 mL.
Samples	1 mL	---	5 mL.
glucose 10 $\mu$ g	10 $\mu$ L	990 $\mu$ L	5 mL.
glucose 20 $\mu$ g	20 $\mu$ L	980 $\mu$ L	5 mL.
glucose 40 $\mu$ g	40 $\mu$ L	960 $\mu$ L	5 mL.
glucose 80 $\mu$ g	80 $\mu$ L	920 $\mu$ L	5 mL.
glucose 100 $\mu$ g	100 $\mu$ L	900 $\mu$ L	5 mL.
glucose 160 $\mu$ g	160 $\mu$ L	840 $\mu$ L	5 mL.

Use tightly stoppered screw capped tubes.

Place tube in a water bath 35° for 45 minutes, keep protected from the light.

Cool to room temperature for 10 minutes in the dark.

Measure the absorbance in 1-cm cells at 505 nm against the water blank.

Read absorbance within 30 minutes.

Subtract the a-amylase reading from the sample reading.

Determine the amount of glucose in the samples by comparing them to the standard curve.

Multiply the amount of glucose in the samples by 0.9 to give an indication of the amount of starch in the samples. This is an adjustment from free glucose to anhydro glucose as occurs in starch.

#### REFERENCES

Karkalas, John, An improved enzymatic Method for the Determination of Native and Modified Starch, J Sci. Food Agric., 36, 1019, 1985.

Amer. Assoc. Cereal Chem. Approved Methods, 9th ed. Method 76-12, 1995.